

Supplemental Information

The *Card19* locus of murine chromosome 13 regulates terminal cell lysis downstream of caspase activation and Gasdermin-D cleavage

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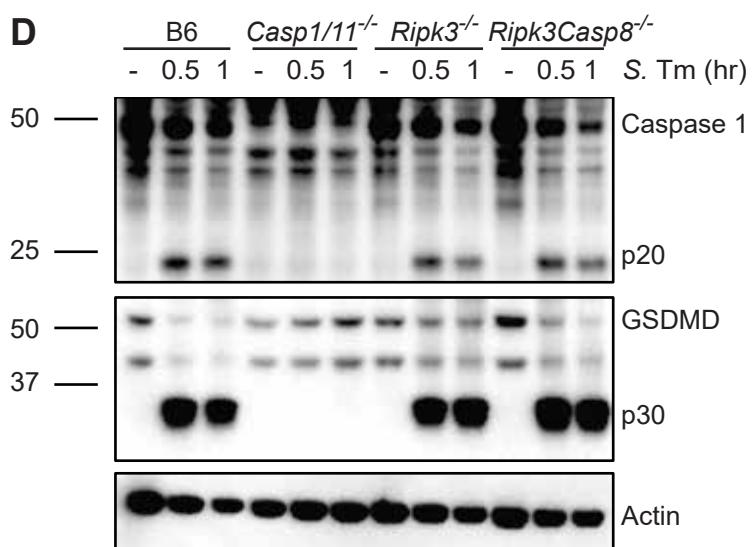
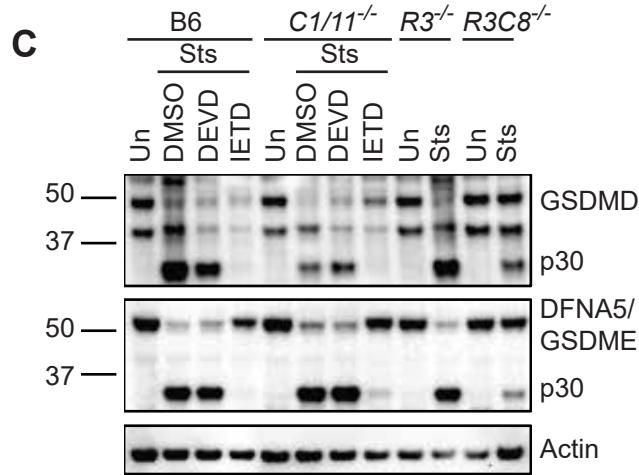
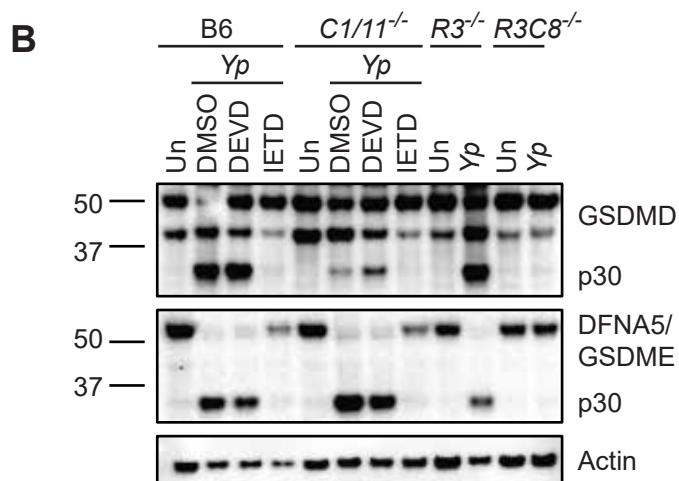
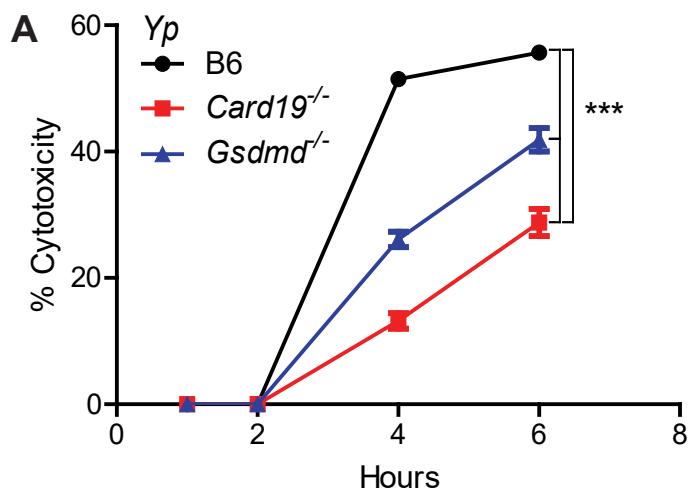
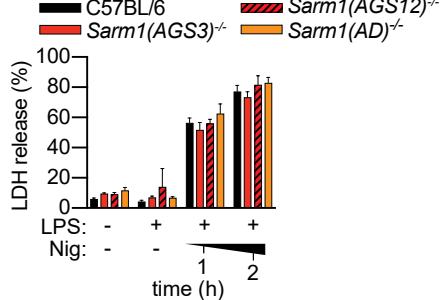
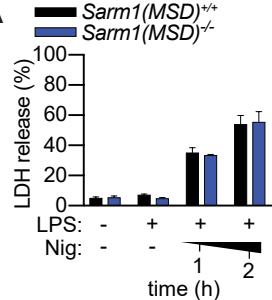


Fig. S1. Card^{-/-} BMDMs are not deficient for Caspase-1 or Caspase-8 activity

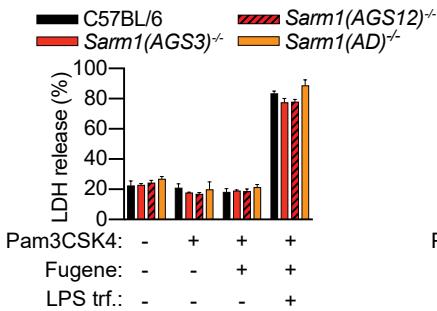
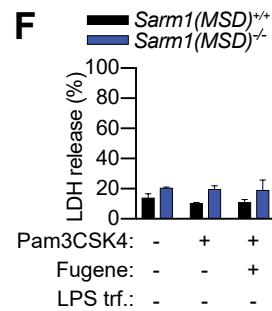
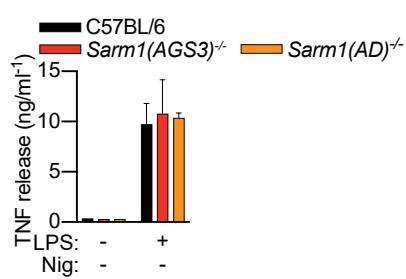
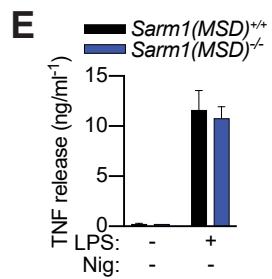
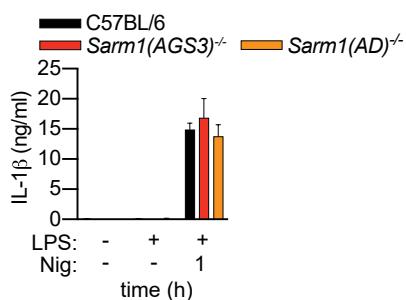
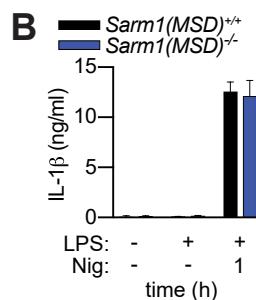
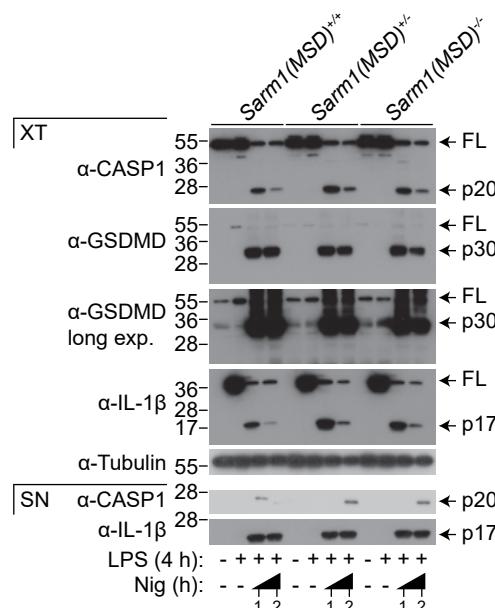
B6, Casp1/11^{-/-}, Ripk3^{-/-}, or Ripk3^{-/-}/Casp8^{-/-} BMDMs were left uninfected (Un) (A) infected with Yp, (B) treated with sts or infected with S. Tm in the presence of DMSO, the caspase-3/7 inhibitor DEVD, and the caspase-8 inhibitor IETD. Lysates were harvested (A, B) 3 hours or (C) 0.5 and 1 hour post treatment and analyzed by western blotting for GSDMD, DFNA5/GSDME, Caspase-8, Caspase-3 and actin (loading control). Blots representative of two or three independent experiments.

(D) B6, Card19^{-/-}, and Gsdmd^{-/-} BMDMS were infected with Yp. Cell death was assayed by LDH release. Representative of three independent experiments.

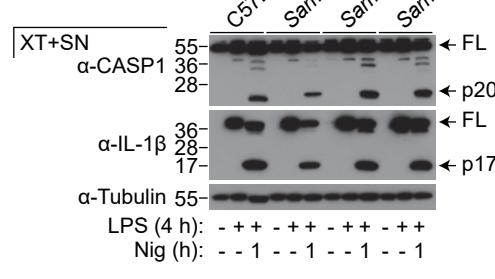
A



C



D



G

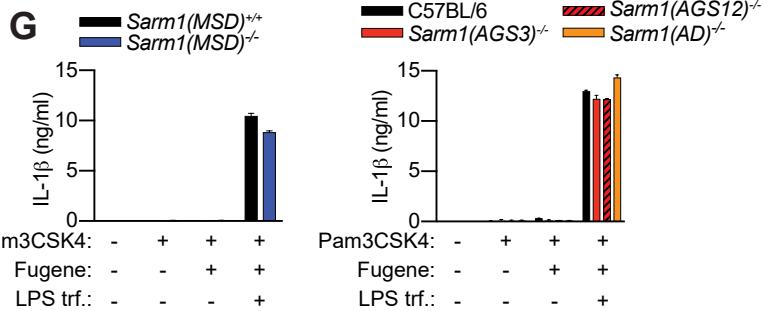
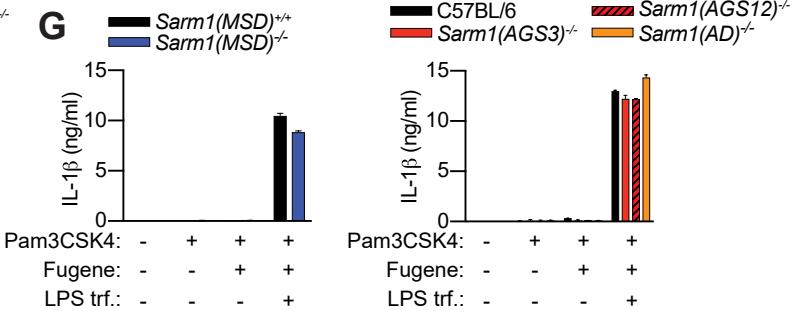


Fig. S2. SARM1 does not regulate canonical and non-canonical inflammasome death and cytokine release

(A to D) *Sarm1(MSD)^{-/-}*, *Sarm1(MSD)^{+/-}*, *Sarm1(MSD)^{+/+}*, C57BL/6, *Sarm1(AGS3)^{-/-}*, *Sarm1(AGS12)^{-/-}* and *Sarm1(AD)^{-/-}* BMDMs were primed with LPS (100 ng/ml) for 4 hours and stimulated with nigericin (5 µM). (A) LDH and (B) IL-1 β release were measured at the indicated time points. (C) Supernatant and cell extract or (D) mixed supernatant and cell extract were examined by immunoblotting at the indicated time points.

(E) BMDMs were primed with LPS (100 ng/ml) and TNF release was measured after 4 hours.

(F and G) BMDMs were primed with Pam3CSK4 (1 µg/ml) for 4 h and were transfected with 2 µg/ml E. coli O111:B4 LPS with Fugene HD. (F) LDH and (G) IL-1 β release were measured after 16 h.

(A and B, E-G) Data are mean + SD of triplicate cell stimulation and is representative of two to four independent experiments. Immunoblots are representative of two independent experiments.

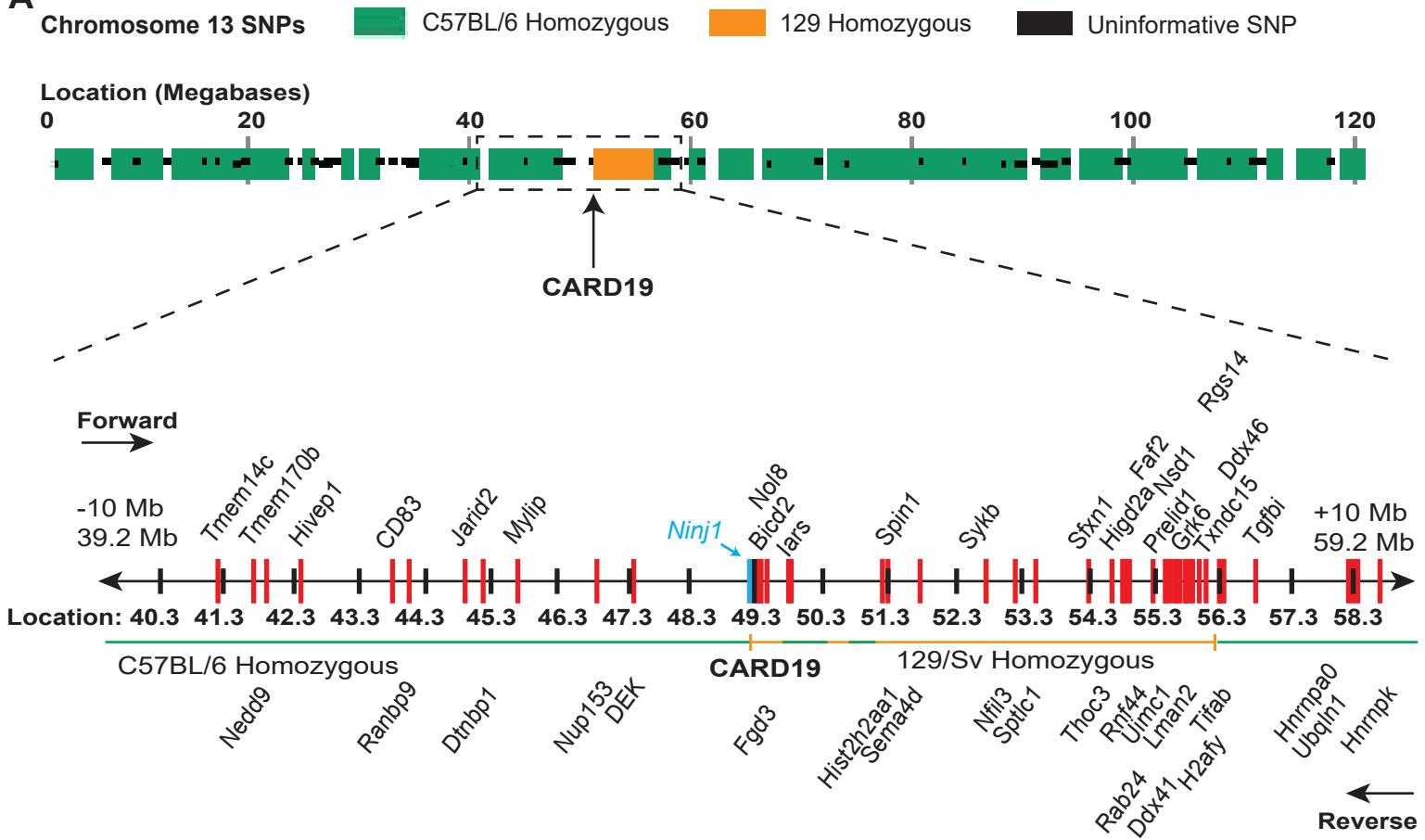
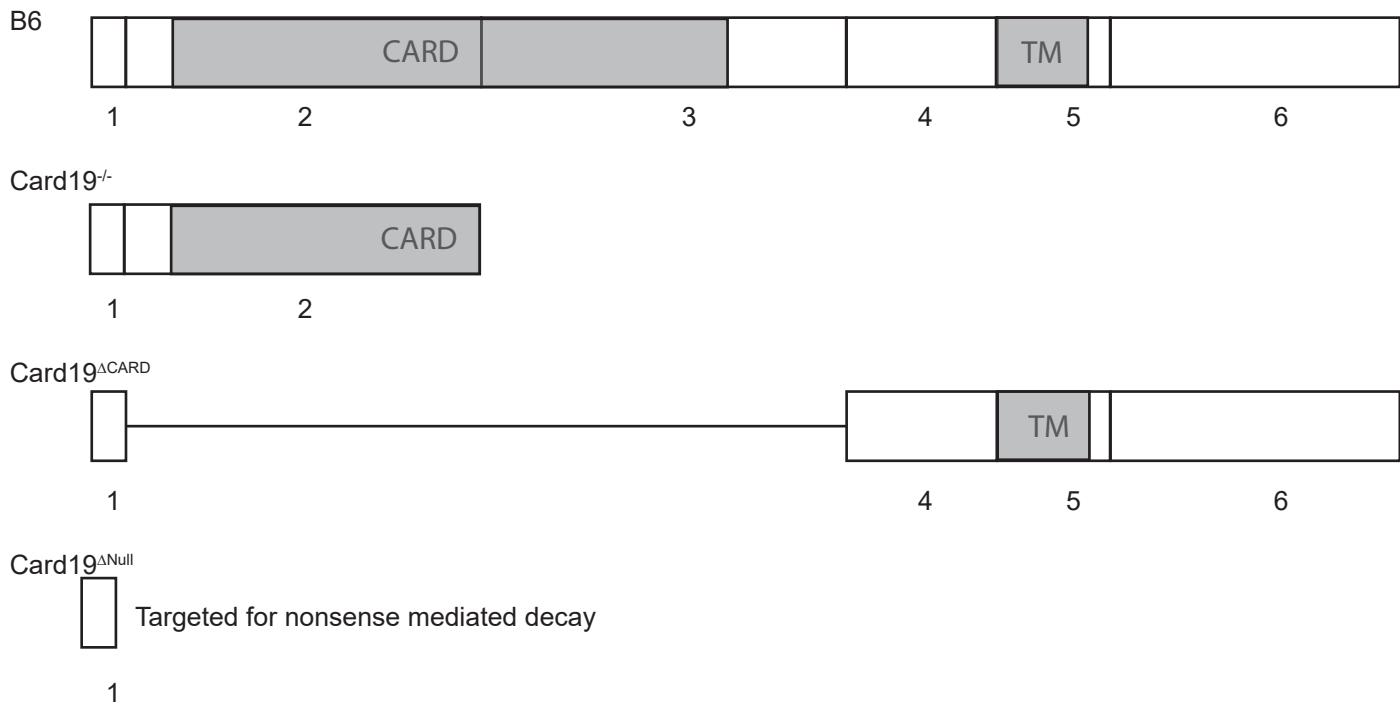
A**B****S3**

Fig. S3: A six megabase region at the *Card19* locus remains homozygous for 129SvEvBrd

(A) Chromosome 13 with tested SNPs from DartMouse genetic background check. C57BL/6 SNPs are in green, 129SvEvBrd SNPs are in yellow, and uninformative SNPs (i.e. not all samples gave identical results) are in black. The 10 megabase region on either side of the *Card19* locus is zoomed in below it with chromosomal locations noted in bold numbers and black notches. Genes in red are expressed in macrophages. *Ninj1* is in blue. The six megabase region highlighted by the yellow bar is homozygous for 129SvEvBrd. The green regions are homozygous for C57BL/6.

(B) CARD19 exon schematic showing each independent CARD19 mouse line with the respective CARD19 product.

Table S1: BMDM and Murine Sources

<u>Macrophages</u>	<u>Mice Generation</u>	<u>References</u>
<i>Card19</i> ^{-/-}	129 ESC and backcrossed to B6	Rios et al. 2020
<i>Card19</i> ^{ACARD}	C57BL/6J CRISPR Line	This paper
<i>Card19</i> ^{null}	C57BL/6J CRISPR Line	This paper
<i>Sarm1(MSD)</i> ^{-/-}	129 ESC and backcrossed to B6	Szrette et al. 2009
<i>Sarm1(AD)</i> ^{-/-}	129 ESC and backcrossed to B6	Kim et al., 2007, JAX stock #018069
<i>Sarm1(AGS3)</i> ^{-/-}	C57BL/6J CRISPR Line	Uccellini et al., 2020, JAX stock #034399
<i>Sarm1(AGS12)</i> ^{-/-}	C57BL/6J CRISPR Line	Uccellini et al., 2020

Table S2: Chromosome 13 SNP Results

<u>SNP</u>	<u>Location</u>	<u>B6/129</u>	<u>Change</u>	<u>Gene ID (ENMUSG)</u>	<u>Gene Name</u>	<u>Function</u>
rs30100204	39337735	B6	intergenic variant			
rs36514425	39958176	B6	intergenic variant			
rs29228586	40345334	B6	intron variant	00000047094	orofacial cleft 1 candidate	associated with cleft lip
rs50931018	42450412	B6	intergenic variant			
rs29236582	42602578	B6	intergenic variant			
rs29635560	42602754	B6	intergenic variant			
rs13481783	42840147	B6	intron variant	00000054728	phosphatase and actin regulator 1	motility and cytoskeletal organization
rs46355744	43000858	B6	intron variant	00000054728		
rs29552103	43005082	B6	intron variant	00000054728		
rs51682551	43032240	B6	intron variant	00000054728		
rs3712907	43171011	B6	intron variant	00000021368	Tbc1d7	cell growth and differentiation
rs29995243	43206944	B6	intron variant	00000051335	glucose-fructose oxidoreductase domain containing 1	
rs29864465	44039283	B6	intergenic variant			
rs46963560	45010065	B6	intergenic variant			
rs6296954	45351934	B6	intergenic variant			
rs3688207	45359288	B6	intergenic variant			
rs108216631	45395035	B6	intron variant	00000038175	Idol/Mir/Myelip	lipid metabolism
rs29225851	46060929	B6	intron variant	00000221550	predicted gene	
rs3663819	46297108	B6	intron variant	00000063529	stathmin domain containing 1	cell differentiation
rs6411274	47129920	B6	intron variant	00000038068	ring finger protein 144B , lbrdc2	E3 ubiquitin ligase
rs29225085	47229758	B6	intron variant	00000038068	ring finger protein 144B , lbrdc2	E3 ubiquitin ligase
rs29914889	47764476	B6	intergenic variant			

rs6244558	47815991	B6	intergenic variant			
rs220959940	48010713	B6	intron variant	00000047324	RIKEN cDNA 4931429P17	
rs29568118	48119885	B6	intron variant	00000097622	RIKEN cDNA A330033J07	
rs30191571	48607862	B6	intron variant	00000038042	protein tyrosine phosphatase domain containing 1	
	49340961			00000037966	Ninjurin 1 (NINJ1)	Terminal Pore Regulation
	49356426			1110007C09Rik	CARD19	
rs37780795	49403959	B6	intergenic variant			
rs6330796	51046190	B6	intergenic variant			
rs226625541	51769402	129	intron variant	00000021451	Sema4d	Signaling
rs46751182	52014198	B6		00000793968		
rs30070966	52018425	129	downstream gene variant	00000102173	predicted gene	
rs29231157	52019172	129	downstream gene variant	00000102173	predicted gene	
rs36690691	52951172	129	intergenic variant			
rs29529592	53233552	129	intron variant	00000021464	ntrk2	
rs49400466	53412842	129	intron variant	00000107008	predicted gene	
rs30058409	53813956	129	intergenic variant			
rs29551959	54964992	129	intron variant	00000025876	unc5h1	
rs30004717	54967613	129	intron variant	00000025876	unc5h1	
rs29927068	55063173	129	intron variant	00000025878	ubiquitin interaction motif containing 1	
rs29239941	56024612	129	intron variant	00000114493	predicted gene	
rs29234727	56057642	129	intron variant	00000114493	predicted gene	
rs29227915	56074012	129	3' UTR variant	00000015937	macroH2A.1 histone	
rs46196633	56077106	129	intron variant	00000015937	macroH2A.1 histone	
rs3720782	56229525	129	intron variant	00000097361	RIKEN cDNA 4930550C17 gene	
rs3700819	57253190	B6	intergenic variant			
rs30059311	57271612	B6	intergenic variant			
rs29249300	57408045	B6	intergenic variant			
rs30078817	57485175	B6	intron variant	00000056222	Ticn1/Spock1	Cell-cell interactions
rs257680153	58007116	B6	intron variant	00000014164	klhl3	Nephron ion transport
rs13481832	58837796	B6	splice region variant	00000055254	neurotrophic tyrosine kinase, receptor, type 2	Neuronal homeostasis and development

rs30250735	59737415	B6	downstream gene variant	00000181528	RIKEN cDNA 4930528D03	
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SNPs from DartMouse SNP genetic background check 10 megabases upstream and downstream of the *Ninj1/Card19* chromosome 13 locus. SNP identification and chromosomal position are indicated. SNPs are indicated as wildtype (B6) or 129SvEvBrd (129). The change, gene ID, gene name, and known function are listed. *Ninj1* and *Card19* are listed for reference.

Table S3: Chromosome 13 Whole Exome Sequencing Results

Gene ID	Gene Name	Position	Change	Function and notes
Spata31	spermatogenesis associated 31	13, 34.21	R645 to stop	spermatogenesis; testis specific
<i>Nlrp4f</i>	NLR family, pyrin domain containing 4F	13, 34.45	E604Q	organelle development
Gm10324	predicted gene 10324	13, 34.51	S310C, Y316H, A363V, K458R, F551Y	testis, placenta specific
2410141K09Rik	RIKEN cDNA 2410141K09 gene	13, 34.52	several intergenic region Indels	testis specific
2410141K09Rik	RIKEN cDNA 2410141K09 gene	13, 34.52	intron variant, 153A>G	testis specific
Adcy2	adenylate cyclase 2	13, 35.55	intergenic region, 6802150A>G	cAMP signaling
Cmya5	cardiomyopathy associated 5	13, 47.81	A3414P	anchoring protein for PKA, skeletal muscle regeneration
Card19		13, 49.35-36		
<i>Naip1</i>	NLR family, apoptosis inhibitory protein 1	13, 53.18	W288L	inhibits apoptosis
Tmem267	transmembrane protein 267	13, 67.25	intron variant, 1131C>T	testis specific

Table S4: RNA-Seq Results: Untreated B6 BMDMs vs. Untreated *Card19*^{-/-} BMDMs

Gene ID	B6 Average	Card19^{-/-} Average	Log Fold Change	Function
<i>Sirt5</i>	0.44	-6.27	-6.71	NAD+ regulation
<i>Ninj1</i>	6.45	4.92	-1.53	Plasma membrane rupture regulation
<i>Axl</i>	6.59	5.51	-1.07	Inhibits TLR signaling
<i>Wdfy1</i>	3.82	4.83	1	Positively regulates TLR signaling
<i>Cxcl14</i>	5.47	7.14	1.67	Chemokine for innate immune cells

Table S5. Key resources and reagents.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-CARD19 polyclonal antibody	Atlas Antibodies	Cat# HPA010990, RRID:AB_2668400
Caspase-1 antibody	Genetech	N/A
Rabbit polyclonal to Caspase-3	Cell Signaling Technologies	Cat# 9662S, RRID:AB_10694681
Rat Anti-Mouse Caspase-8 Monoclonal Antibody, Clone 1G12	Enzo Life Technologies	Cat# ALX-804-447-C100, RRID:AB_2050952
Anti-DFNA5/GSDME antibody [EPR19859] – N-terminal	Abcam	Cat# ab215191, RRID:AB_2737000
Rabbit monoclonal [EPR19828] to GSDMD	Abcam	Cat# ab209845

Rabbit Polyclonal to HDAC1	Cell Signaling Technologies	Cat# 2062, RRID:AB_2118523
HA-Tag (C29F4) Rabbit mAb	Cell Signaling	Cat#3724T
HMGB1 antibody - ChIP Grade	Abcam	Cat# ab18256, RRID:AB_444360
Purified Mouse anti-Ninjurin clone 50	BD Biosciences	Cat# #610776, RRID: AB_398097
Monoclonal Anti-alpha-Tubulin antibody produced in mouse	Sigma-Aldrich	Cat# T5168, RRID:AB_477579
Goat Anti-Rabbit IgG (H+L) Antibody, Alexa Fluor 488 Conjugated	Molecular Probes	A-11008, RRID:AB_143165
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 514	Thermo Fisher Scientific	Cat# A-31555, RRID:AB_2536171
Alexa Fluor® 647 Phalloidin antibody	Thermo Fisher Scientific	Cat# A22287, RRID:AB_2620155
Mouse Anti-beta-Actin Monoclonal Antibody, Unconjugated, Clone AC-74	Sigma-Aldrich	Cat# A2228, RRID:AB_476697
Peroxidase-AffiniPure Goat Anti-Rat IgG (H+L) (min X Hu,Bov,Hrs,Rb Sr Prot) antibody	Jackson Labs	Cat# 112-035-143, RRID:AB_2338138
Peroxidase-AffiniPure Goat Anti-Rabbit IgG (H+L) (min X Hu,Ms,Rat Sr Prot) antibody	Jackson Labs	Cat# 111-035-144, RRID:AB_2307391
Anti-mouse IgG, HRP-linked Antibody	Cell Signaling Technologies	Cat# 7076, RRID:AB_330924
IL-1a antibody	BD Biosciences	Cat# 550604, RRID:AB_393776
Biotin anti-mouse IL-1a antibody	BioLegend	Cat# 512504, RRID:AB_2124220
Anti-Mouse/Rat IL-1 beta Purified 500 ug antibody	Thermo Fisher Scientific	Cat# 14-7012-85, RRID:AB_468397
Anti-Mouse IL-1 beta Biotin 500 ug antibody	Thermo Fisher Scientific	Cat# 13-7112-85, RRID:AB_466925
Rat Anti-IL-6 Monoclonal Antibody, Unconjugated, Clone MP5-20F3	BD Biosciences	Cat# 554400, RRID:AB_398549
Rat Anti-IL-6 Monoclonal Antibody, Biotin Conjugated, Clone MP5-32C11	BD Biosciences	Cat# 554402, RRID:AB_395368
Rat Anti-IL-12 (p40 / p70) Monoclonal Antibody, Unconjugated, Clone C15.6	BD Biosciences	Cat# 551219, RRID:AB_394097
Rat Anti-IL-12 (p40 / p70) Monoclonal Antibody, Biotin Conjugated, Clone C17.8	BD Biosciences	Cat# 554476, RRID:AB_395419
IFN gamma Monoclonal Antibody (AN-18), eBioscience(TM)	Thermo Fisher Scientific	Cat# 14-7313-85, RRID:AB_468472
IFN gamma Monoclonal Antibody (R4-6A2), Biotin, eBioscience(TM)	Thermo Fisher Scientific	Cat# 13-7312-85, RRID:AB_466939
Goat anti-Mouse IgG1 Secondary Antibody, Alexa Fluor 488 conjugate	Thermo Fisher Scientific	Cat# A-21121, RRID:AB_2535764
Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat# A-21240, RRID:AB_2535809
Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21131, RRID:AB_2535771
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Thermo Fisher Scientific	Cat# A-21245, RRID:AB_2535813
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 546	Thermo Fisher Scientific	Cat# A-11010, RRID: AB_143156
Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 546	Thermo Fisher Scientific	Cat# A-11003, RRID: AB_2534071
Bacterial and Virus Strains		
<i>Yersinia pseudotuberculosis</i> IP2777	Stanley Falkow	(1)
<i>Yersinia pseudotuberculosis</i> IP2666	Jim Bliska	(2)
<i>Yersinia pseudotuberculosis</i> IP2666 ΔyopEJK	Erin Zwack, Igor Brodksy	(3)
<i>Salmonella</i> Typhimurium SL1344	(4)	

<i>ΔoatA</i> <i>Staphylococcus aureus</i>	Jonathan Kagan	(5)
<i>E. coli</i> DH5α	N/A	
ER-HoxB8 Virus	David Sykes	(6)
Biological Samples		
<i>Gsdmd</i> ^{-/-} Bone Marrow	Russell Vance	(7)
<i>Mavs</i> ^{-/-} Bone Marrow	Carolina Lopez, Jackson Labs	Cat# 008634
<i>Casp11</i> ^{-/-} Bone Marrow	Junying Yuan	(8)
<i>Sarm1(AGS3)</i> ^{-/-} , <i>Sarm1(AGS12)</i> ^{-/-} , <i>Sarm1(AD)</i> ^{-/-} Bone Marrow	Adolfo Garcia-Sastre	(9)
Chemicals, Peptides, and Recombinant Proteins		
Hoechst	Thermo Fisher Scientific	Cat# 62249
Fluormount G	Thermo Fisher Scientific	Cat# 00-4958-02
Lipopolysaccharide from E Coli	Sigma-Aldrich	Cat# L2880
Pam3CSK4	Invivogen	Cat# tirl-pms
Cycloheximide from microbial	Sigma-Aldrich	Cat# C7698
zVAD(Ome)-FMK	SM Biochemicals	Cat# SMFMK001
z-IETD-FMK	SM Biochemicals	Cat# SMFMK004
Adenosine 5'-Triphosphate, Disodium Salt	Millipore	Cat# 1191
Gentamicin Sulfate	Sigma-Aldrich	Cat# G1914
Irgasan	Sigma-Aldrich	Cat# 72779
Streptamycin Sulfate	Gold Technologies	Cat# G-400-1
Staurosporine from <i>Streptomyces</i> sp.	Sigma-Aldrich	Cat# S5921
Necrostatin (Nec-1)	EMD Chemicals	Cat# 480065
MitoTracker CMXRos	Life Technologies	Cat# M7512
Propidium iodide	Thermo Fisher Scientific	Cat# P3566
ECL Western Blotting Substrate	Thermo Fisher Scientific	Cat# 32106
SuperSignal West Femto Maximum Sensitivity Substrate	Thermo Fisher Scientific	Cat# 34095
Complete, Mini, EDTA-Free Protease Inhibitor Cocktail	Roche	Cat# 11836170001
Streptavidin HRP	BD Biosciences	Cat# 554066
Recombinant Mouse TNF-alpha	BioLegend	Cat# 575206
Recombinant Mouse IFN-gamma protein	eBioscience	Cat# 485-MI-100
Mouse IL-1beta Recombinant Protein	R&D	Cat# 14-8012-80
Recombinant IL-6 Standard	R&D	Cat# 406-ML-005
Recombinant Mouse IL-12 protein	R&D	Cat# 419-ML-010
Recombinant Mouse IL-1 alpha	R&D	Cat# 400-ML-005
Lipofectamine 2000	Invitrogen	Cat# 11668027
16% Paraformaldehyde Aqueous Solution, EM Grad	Electron Microscopy Services	Cat# 15710
Mounting Media, PPD in 90% Glycerol	(10)	
DAPI	Molecular Probes	Cat# D-1306
Todd Hewitt Broth for microbiology	Sigma-Aldrich	Cat# T1438
AP20187	ApexBio	Cat# B1274
Commercial Assays		
Plasma Membrane Protein Extraction Kit	Abcam	Cat# ab65400
LDH Cytotoxicity Detection Kit	Takara Bio	Cat# MK401
CellTiter Glo Luminescent Cell Viability Assay	Promega	Cat# G7571
Plasmid Maxiprep Kit	Qiagen	Cat# 12162
Caspase-Glo 8 Assay	Fisher Scientific	Cat# PRG8201
Experimental Models: Cell Lines		
HEK 293T Cells	ATCC	
Experimental Models: Organisms/Strains		
<i>Card19</i> ^{-/-} Mice	Brian Schaefer	(11)

<i>Card19</i> ^{ACARD} Mice	This paper	
<i>Card19</i> ^{Null} Mice	This paper	
C57BL/6J Mice (B6)	Jackson Labs	Cat# 000664
<i>Casp1/Casp11</i> ^{-/-} Mice	Jackson Labs	Cat# 016621
<i>Ripk3/Casp8</i> ^{-/-} Mice	Doug Green	(12)
<i>Sarm1(MSD)</i> ^{-/-}	Adriano Aguzzi	(13)
<i>Ripk3</i> ^{-/-} Mice	Kim Newton, Vishva Dixit	(14)
Oligonucleotides		
<i>Card19</i> ^{-/-} Wt genotyping primers, (297 bp) CATGGATGTACAGAGCTCGGTA, CGTTGCCCTGGAGACACAGTATT	IDT	This paper
<i>Card19</i> ^{-/-} Knockout genotyping primers (281 bp) CGGAATTGATCCCGCTCGAA, CGTTGCCCTGGAGACACAGTATT	IDT	This paper
<i>Card19</i> ^{ACARD} Sequencing Primer (250 bp) CTTGGGAAAAGTGTGGCTTTGT, TCCTCCAGTCTGCCATGTGGGGATTT	Sigma	This paper
<i>Card19</i> ^{Null} Sequencing primer (298 bp) TCGGTTCTTCATCCAGGAG, GAGGCAGCCACTGGGTATAA	Sigma	This paper
Recombinant DNA		
pcDNA3.1+/CARD19-FLAG	GenScript	Cat# OMu021914D
pcDNA3.1+	Igor Brodsky	
pMSCV2.2	Igor Brodsky	
pMSCV2.2/CARD19	This paper	
pCL-Eco	Igor Brodsky	
Software and Algorithms		
FIJI	(15)	https://imagej.net/Fiji/Downloads
Volocity 6.3	PerkinElmer	http://cellularimaging.perkinelmer.com/downloads/detail.php?id=14
Prism 5.0	GraphPad	https://www.graphpad.com/scientific-software/prism/
R version 4.0.3	R	https://www.r-project.org/
RStudio version 1.2.5042	RStudio	https://rstudio.com/
Other		

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